

EFFECTS OF MOLYBDENUM AND DIFFERENT SOURCES OF NITROGEN ON THE GROWTH AND CHEMICAL COMPOSITION OF *DIGITARIA SMUTSII* STENT

J. A. DE BRUYN AND J. AALBERS¹

(Department of Botany, University of Stellenbosch, Stellenbosch, South Africa)

ABSTRACT

Plants of *Digitaria smutsii* Stent (Northam ecotype) were grown in solution culture for 13 weeks with two sources of nitrogen, viz., nitrate and ammonium, both with and without molybdenum.

Plants supplied with nitrate plus Mo grew better than those without Mo, or than those supplied with ammonium. The dry weight of the shoots was by far the highest with nitrate plus Mo; there were no significant differences among the other treatments. Root growth was much better with nitrate than with ammonium, and was not affected by the presence or absence of Mo.

The concentration of nitrogen in the shoots tended to be lower with ammonium than with nitrate, and to decrease when Mo was withheld, although differences were not always significant. The N content of the roots was not significantly affected by Mo, but tended to be slightly lower with ammonium than with nitrate. When Mo was supplied, the Mo contents of the shoots and roots were significantly higher with ammonium than with nitrate as source of nitrogen.

The chlorophyll content of the leaves was the highest in plants supplied with nitrate plus Mo, and the lowest in those receiving nitrate only. With ammonium-N in the medium, Mo had no effect upon chlorophyll content.

In general, the levels of free amino acids in the shoots tended to be higher with ammonium than with nitrate in the medium. Alanine appeared to be the principal amino acid in both shoots and roots; it was not affected by nutrient treatment. Asparagine accumulated in shoots and roots when ammonium was supplied, but was absent in plants receiving nitrate. The levels of proline and serine were increased in the presence of Mo, regardless of the source of nitrogen.

Sucrose was present in plants receiving nitrate, but absent from those supplied with ammonium. In the presence of nitrate, glucose and fructose decreased when Mo was withheld, but in the presence of ammonium, Mo had the opposite effect.

It was concluded that *Digitaria smutsii* prefers nitrate to ammonium, and that it requires Mo for optimal growth in the presence of nitrate.

INTRODUCTION

Molybdenum has been shown to be essential for a wide range of plants, among which a number of grass species, e.g., *Avena sativa*, *Dactylis glomerata* and *Lolium perenne* (Hewitt, 1956; Stout and Johnson, 1956). This micronutrient element appears to be involved in the fixation of nitrogen in legumes (Mulder,

¹ Present address: Sea Point Boys' High School, Sea Point, Cape Town.

1948; Nason and McElroy, 1963), as well as in the reduction of nitrates to nitrites in plants (Nicholas and Nason, 1954; Evans, 1956).

Probably because of its role in the reduction of nitrate, a deficiency of Mo usually results in an accumulation of nitrates in plant tissues (Mulder, 1948; Agarwala and Hewitt, 1955a). Molybdenum may also affect other aspects of plant metabolism, e.g., the levels of the various free amino acids (Hewitt, Jones and Williams, 1949), the synthesis of chlorophyll (Hewitt and McCready, 1956), and the concentrations of ascorbic acid (Agarwala and Hewitt, 1955b) and sugars (Agarwala and Hewitt, 1955a) in plants.

The effects of a deficiency of Mo often appear to be less severe in plants receiving ammonium than in those supplied with nitrate (Hewitt, 1963). This is probably a consequence of its role in the reduction of nitrate. In general, however, plants seem to grow better with nitrate than with ammonium as the sole source of nitrogen, although many factors, e.g., the plant species, pH and aeration of the culture solutions may affect the relative merit of these two sources of nitrogen (Pardo, 1935).

Digitaria smutsii Stent is a perennial grass species which is sometimes used in pastures in South Africa. Relatively little is known about its mineral requirements; nothing is known about the effects of the different elements, particularly micronutrient elements, upon its metabolism. An investigation was therefore carried out to study the effects of molybdenum, in combination with two sources of nitrogen, viz., nitrate and ammonium, upon the growth and certain chemical constituents of this species.

MATERIAL AND METHODS

Seeds of *Digitaria smutsii* Stent, Northam ecotype, were germinated in acid-washed quartz sand and supplied with deionised water. When the seedlings were about 2 to 3 cm. tall, they were transferred to solution culture in 600 ml. polyethylene containers, aerated twice daily for one hour. Four treatments were applied, viz.: nitrate plus Mo; nitrate minus Mo; ammonium plus Mo; and ammonium minus Mo. All the nutrient solutions contained 140 ppm. of nitrogen. Six replicates of each treatment were used; they were arranged in randomised blocks.

The solutions were composed as follows:

Nitrate-nitrogen solution: 4 mM Ca $(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 2 mM CaCl_2 ; 2 mM KNO_3 ; 1 mM K_2SO_4 ; 2 mM KH_2PO_4 ; 2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Ammonium-nitrogen solution: 3 mM CaCl_2 ; 2.5 mM K_2SO_4 ; 2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 2 mM $(\text{NH}_4)_2\text{SO}_4$; 3 mM $(\text{NH}_4)_2\text{HPO}_4$.

In addition, each solution contained 1.1 ppm Fe (as NaFe-EDTA), 0.5 ppm B (as H_3BO_3), 0.5 ppm Mn (as MnSO_4), 0.05 ppm Zn (as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), and

0.02 ppm Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). The respective plus-molybdenum solutions also contained 0.01 ppm Mo, as $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$. The pH of the solutions was adjusted to pH 6.5 with N NaOH.

In order to minimize contamination by molybdenum, all solutions were made up in Pyrex glassware and stored in polyethylene containers. Glassware was thoroughly cleaned in potassium dichromate-sulphuric acid solution and repeatedly rinsed, first with tap water and then with deionised water. Deionised water (tap water passed through a mixed-bed ion exchange column) was used in all solutions. Salts of the macroelements were purified of heavy metals by co-precipitation with cupric sulphate (Hewitt, 1952).

The plants were grown under conditions of natural daylength in a greenhouse maintained at a temperature not exceeding 30°C. All plants which failed to grow were replaced by others, until all were properly established. The experiment commenced on the 13th March, 1963; all plants were harvested at the age of 13 weeks. Nutrient solutions were renewed weekly for the first three weeks, and twice weekly after that. On intervening days the solutions were supplemented with deionised water.

At harvest time the tillers of each plant were counted. The aerial parts (shoots) and roots were then separated. Plants of four replicates were dried at 100°C for 48 hours and their dry weights determined. They were subsequently used for analyses of nitrogen and molybdenum. Plants of the remaining two replicates were placed in polyethylene bags and immediately stored in a freezer at -9°C; they were later used for the determination of chlorophyll, free amino acids, and sugars.

The nitrogen content of the plants was determined by the micro-Kjeldahl method (Humphries, 1956). Molybdenum was analysed according to the ammonium thiocyanate method (Prince, 1955); colour comparisons were made at 500 nm. with the aid of a Klett-Summerson photoelectric colorimeter. The chlorophyll *a* and *b* contents of the leaves were determined spectrophotometrically according to the method of MacKinney (1941), using a Beckman DB spectrophotometer.

Free amino acids and sugars were extracted from the shoots and roots by grinding 10 g. fresh material with sufficient absolute ethanol to give a final concentration of 80% ethanol, filtering and washing the residue with 50 ml. 80% ethanol. Chlorophyll and other pigments were removed from the leaf extracts by shaking the combined filtrate and washings with 200 ml. chloroform; the upper, aqueous layer was used.

Amino acids were removed from the extract by passing it through a column of Amberlite IR-120 resin (H^+ form). The amino acids were eluted from the resin with the aid of N NH_4OH , evaporated to dryness under reduced pressure

at 40°C and the residue dissolved in 1.0 ml. 10% isopropanol. The solution which had passed through the column, and which contained the sugars and other materials, was also evaporated to dryness under reduced pressure at 40°C, the residue dissolved in 5 ml. redistilled pyridine at 100°C, filtered and again evaporated to dryness. The resulting residue was dissolved in 1.0 ml. 10% isopropanol.

The free amino acids were separated by means of two-dimensional descending chromatography on Whatman No. 1 paper, with phenol-water (80:20; v/v) as the first and 1-butanol- acetic acid- water (4:1:1; v/v) as the second solvent system. Their positions were located by spraying the chromatograms with 0.25% ninhydrin in acetone. The different amino acids were identified by comparison with standard charts, or by co-chromatography.

Sugars were separated by means of one-dimensional descending chromatography on Whatman No. 1 paper, with phenol-water (80:20; v/v) as solvent. They were located by spraying the chromatograms first with a solution of 2 ml. aniline plus 100 ml. ethyl acetate containing 2% trichloroacetic acid, drying them at 80–85°C, and then spraying them with a 1% resorcinol solution. The different sugars were identified by comparing them with standard compounds on the same paper.

The relative concentrations of the different amino acids and sugars were estimated according to the size and intensity of the spots produced on the chromatograms, and graded into five groups, from 0 to 4.

The results obtained from the first four replicates (i.e., growth measurements and determinations of mineral content) were analysed statistically according to standard methods of analysis of variance (Snedecor, 1956). Results in respect of chlorophyll content and the levels of free amino acids and sugars were not statistically analysed.

RESULTS AND DISCUSSION

General observations on growth

All the plants supplied with nitrate were readily established, but considerable difficulty was encountered in establishing those plants receiving ammonium, particularly when Mo was withheld.

After two weeks the leaves of the plants receiving nitrate without Mo developed signs of chlorosis, which could not be alleviated by increasing the application of iron. After eight weeks these plants were markedly yellowish-green, in contrast to the dark green leaves of the plants receiving nitrate plus Mo. The plants receiving ammonium also appeared somewhat chlorotic. At this time (eight weeks) some leaves of all the plants began to show a reddish-purple discoloration, followed by progressive necrosis. This phenomenon was

much more marked in the plants receiving ammonium than in those supplied with nitrate; it did not appear to be related to the supply of molybdenum.

In general, the plants receiving Mo seemed to grow better than those without Mo, particularly when they were supplied with nitrate. Plants supplied with nitrate appeared to grow better than those with ammonium; the roots of the former were healthy, white and much better developed than those of the latter treatment, which were yellowish-brown and rather spindly.

During the course of the experiment inflorescences developed only in four plants: two receiving nitrate plus Mo and two supplied with ammonium minus Mo. The latter two ears died without maturing. The poor development of ears might have been related to the fact that the experiment was carried out during the autumn. Apart from these, one could not distinguish any effects of treatment upon the development of the plants.

Growth results

Best growth of the shoots (in terms of dry weight as well as tiller production) occurred in the plants supplied with nitrate plus Mo, with no significant differences among the other treatments (Table 1). This means that the growth of plants supplied with nitrate was reduced when Mo was withheld, and when plants received a complete nutrient (i.e., including Mo) they grew better with nitrate than with ammonium. The shoot growth of plants supplied with ammonium was not affected by the presence or absence of Mo.

Agarwala and Hewitt (1955b), Hewitt and McCready (1956) and Minina (1960) found the dry weights of shoots of cauliflower, tomatoes and lettuce respectively to be increased upon the addition of Mo, regardless of the source of nitrogen. In the presence of Mo, all these authors obtained better growth with nitrate alone than with ammonium, but in the absence of Mo the yields were higher with ammonium than with nitrate. All three species (cauliflower, tomato and lettuce) gave the lowest yields in the presence of nitrate alone.

The differences between our results and those of the above mentioned authors might indicate that *D. smutsii* is more sensitive than cauliflower, tomato or lettuce to ammonium nitrogen. On the other hand, *D. smutsii* was grown in solution culture, whereas these plants were grown in sand or soil, which was probably better aerated, with the result that they might have been better able to utilise the ammonium.

Molybdenum had no significant effect upon root growth, regardless of the source of nitrogen (Table 1). Root growth both with and without Mo was much poorer with ammonium than with nitrate. These results are also reflected in the much higher top:root ratio in plants supplied with ammonium

than with nitrate. The fact that, with nitrate as source of nitrogen, the absence of Mo retarded top growth but not root growth, is also reflected in the top:root ratios of the plus-Mo and minus-Mo treatments.

TABLE 1
Influence of nitrogen source and molybdenum upon number of tillers, dry weights of shoots and roots, and top/root ratio

Treatment	Number of tillers per plant	Dry wt. of shoots g	Dry wt. of roots g	Top/root ratio D. W. basis
Nitrate + Mo ..	120.5	37.8	7.0	5.5
Nitrate - Mo ..	80.8	11.4	6.3	1.8
Ammonium + Mo	87.5	10.9	0.8	13.6
Ammonium - Mo	72.5	10.0	0.7	14.3
L.S.D. (P = 0.05)	31.2	19.2	3.2	— a)

a) Not calculated.

The foregoing results indicate that *D. smutsii* prefers nitrate to ammonium as a source of nitrogen. In this respect it reacts similarly to barley, oats and rye (Pardo, 1935). Although the two nutrient solutions also differed in other respects than the source of nitrogen (i.e., the plants supplied with ammonium received less Ca and K, and more P, S and Cl than those supplied with nitrate), it is doubtful whether these differences in composition would have had an appreciable effect upon growth.

It further appears that, when nitrate is supplied, the plants respond to Mo, particularly where the growth of the shoots is concerned. The fact that *D. smutsii* responded to a lesser degree to Mo in the presence of ammonium, might probably be related to the role of Mo in the reduction of nitrate. In addition, the apparently deleterious effects of the ammonium treatment might have masked a possible response to Mo, since Hewitt (1963) has concluded that Mo might have a multiple role not yet fully elucidated.

Nitrogen and molybdenum contents

The effects of the different treatments on the nitrogen content of the shoots and roots are summarised in Table 2. Although the nitrogen concentration in the shoots tended to be higher where nitrate had been supplied than with ammonium, this difference was significant only in the presence of Mo. In plants receiving ammonium, the nitrogen concentration was lower with than without Mo. When nitrate was supplied, however, Mo had no significant

effect upon the nitrogen content of the shoots. When one calculates the total nitrogen in the shoots, it is clear that the plants supplied with nitrate plus Mo contained the most nitrogen, and those receiving ammonium plus Mo the smallest total amount of nitrogen.

TABLE 2
Influence of nitrogen source and molybdenum on nitrogen content of shoots and roots

Treatment	N content of shoots		N content of roots	
	% of dry wt.	mg. per plant	% of dry wt.	mg. per plant
Nitrate + Mo	0.97	366	1.22	85
Nitrate - Mo	1.06	121	1.21	76
Ammonium + Mo	0.52	57	1.06	8.5
Ammonium - Mo	0.93	93	0.93	6.5
L.S.D. (P = 0.05)	0.18	—a)	0.27	—a)

a) Not calculated.

The nitrogen concentration in the roots of plants receiving nitrate tended to be higher than those with ammonium, but the differences were significant only in the absence of Mo (Table 2). Molybdenum itself had no significant effect upon the nitrogen content of the roots. The total nitrogen content of the roots, like that of the shoots, was considerably higher in the presence of nitrate than with ammonium; Mo probably had no significant effect in this respect.

It would appear, therefore, that the plants tended to absorb more nitrogen (per unit of dry weight) when supplied with nitrate than with ammonium, whereas Mo had no consistent effect upon the concentration of nitrogen in the tissues.

The nitrogen contents reported here represents only total nitrogen, and gives no indication of the nitrate content of the tissues. A number of authors, e.g., Agarwala and Hewitt (1955b) and Minina (1960) have found considerably more nitrate-nitrogen in plants supplied with nitrate in the absence of Mo than in its presence.

The effects of the different treatments upon the Mo content of the shoots and roots are summarised in Table 3. As could be expected, the addition of Mo resulted in considerable increases in Mo content. When Mo was withheld, the source of nitrogen had no effect upon the concentration of Mo, but with Mo

in the medium the concentration of Mo in the tissues tended to be higher where ammonium had been supplied than with nitrate. The Mo level with any particular treatment was more or less the same in the shoots and in the roots; it was more or less the same as found in other members of the Gramineae under fairly similar conditions (Stout and Johnson, 1956).

TABLE 3
Influence of nitrogen source and molybdenum on molybdenum
content of shoots and roots

Treatment	Mo content ($\mu\text{g/g}$ dry wt.)	
	Shoots	Roots
Nitrate + Mo	0.05	0.06
Nitrate - Mo	0.01	0.01
Ammonium + Mo	0.07	0.08
Ammonium - Mo	0.02	0.01
L.S.D. (P = 0.05)	0.02	0.02

Chlorophyll content of the leaves

Although the results (Table 4) only represent the averages of duplicate determinations, it appears that the plants supplied with nitrate plus Mo had the highest, and those receiving nitrate without Mo had the lowest chlorophyll content. The high chlorophyll content of the former appears to result from increased levels of both chlorophyll *a* and *b*, with relatively more chlorophyll *b*. With nitrate as a source of nitrogen, a decreased supply of Mo therefore resulted

TABLE 4
Influence of nitrogen source and molybdenum on chlorophyll content of leaves (in mg.
per g fresh material)

Treatment	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Ratio a : b
Nitrate + Mo ..	.. 0.70	0.41	1.11	1.7
Nitrate - Mo ..	.. 0.40	0.10	0.50	4.0
Ammonium + Mo	.. 0.55	0.13	0.68	4.2
Ammonium - Mo	.. 0.52	0.16	0.68	3.3

in reduced synthesis of both chlorophyll *a* and *b*. With ammonium in the medium, Mo apparently had no effect upon chlorophyll content. Hewitt and McCready (1956), Agarwala and Hewitt (1955b) and Minina (1960) also found that, with nitrate in the medium, absence of Mo resulted in a decreased level of chlorophyll in the leaves of tomato, cauliflower and lettuce, respectively. With ammonium however, Agarwala and Hewitt (1955b) and Hewitt and McCready (1956) found the chlorophyll content to be increased in the absence of Mo. On the basis of these results Hewitt and McCready (1956) concluded that Mo does not appear to be essential for chlorophyll formation in tomato or cauliflower, provided that nitrogen is available in a form that does not directly involve the mediation of nitrate reductase for its assimilation. Our results may be interpreted in the same way.

TABLE 5
Influence of nitrogen source and molybdenum on relative concentrations* of free amino acids in shoots and roots

Amino acid	Shoots				Roots			
	Nitrate-N + Mo - Mo		Ammonium-N + Mo - Mo		Nitrate-N + Mo - Mo		Ammonium-N + Mo - Mo	
γ -Alanine	4	4	4	4	4	4	3	3
γ -Aminobutyric acid	1	1	2	1	2	1	1	1
Asparagine	0	0	3	3	0	0	2	2
Aspartic acid	3	1	2	3	3	1	2	2
Glutamine	1	1	0	0	1	1	1	1
Glutamic acid ..	2	1	4	4	3	0	3	3
Glycine	2	2	4	4	2	4	2	2
Leucine/isoleucine ..	1	1	3	1	1	1	1	1
Proline	1	0	2	1	1	0	1	0
Serine	1	0	3	1	3	0	3	1
Threonine	2	2	3	3	3	3	3	3
Valine	1	1	3	2	2	2	1	1

* Relative concentrations estimated according to size and intensity of ninhydrin spots, and graded from 0 to 4 as follows: 0—absent; 1—trace; 2—small spot; 3—medium-sized spot; 4—large and intensely coloured spot.

Amino acid content of shoots and roots

The relative amounts of free amino acids in the shoots and roots are listed in Table 5. Although these results are only semiquantitative, they do indicate certain trends. Most of the free amino acids in the shoots appear to have been present in higher concentrations with ammonium than with nitrate; in the roots the source of nitrogen did not seem to have much effect upon the free amino acids. α -Alanine appears to have been the principal amino acid in both shoots and roots; apparently it was not affected by either the nitrogen source or by molybdenum. Asparagine was present only in plants receiving ammonium nitrogen. Glutamine was absent from the shoots of plants receiving ammonium; otherwise it was present only in low amounts. Apparently neither of these two amides was affected by Mo. In the shoots of plants supplied with nitrate the levels of glutamic acid and aspartic acid appeared to decrease when Mo was withheld. Similar results were obtained by Hewitt and Williams (1952) in cauliflower and by Possingham (1956) in tomatoes. Proline and serine were lower in plants without Mo, regardless of the source of nitrogen; in those plants receiving nitrate without Mo they appeared to be completely absent. Steinberg (1956) also observed substantial decreases in the levels of proline and serine in tobacco plants supplied with nitrate and without molybdenum.

TABLE 6
Influence of nitrogen source and molybdenum on relative concentrations* of free sugars in shoots and roots

Sugar		Nitrate-N		Ammonium-N	
		+ Mo	- Mo	+ Mo	- Mo
<i>Shoots</i>					
Fructose	..	..	4	1	2
Glucose	..	..	2	1	2
Sucrose	..	..	1	0	0
<i>Roots</i>					
Fructose	..	..	3	2	4
Glucose	..	..	2	1	4
Sucrose	..	..	1	0	0

* For explanation, see subscript to Table 5.

Sugar content of shoots and roots

The levels of free sugars found in the plants are summarised in Table 6. Again, these results are only semiquantitative, but certain trends could be noted. Sucrose was absent from plants receiving ammonium, but present in

those with nitrate. In plants receiving nitrate, the levels of glucose and fructose were apparently reduced in the absence of Mo, whereas the opposite effect was observed in plants supplied with ammonium. Agarwala and Hewitt (1955a) noted a decrease in the concentrations of total and reducing sugars in cauliflower plants suffering from a deficiency of molybdenum, irrespective of the source of nitrogen. As regards our own results, it is difficult if not impossible to offer a good interpretation of the effects of the different treatments upon the levels of the free sugars.

CONCLUSIONS

Although some of the results obtained were fairly variable—perhaps more replicates should have been used—they have shown that *D. smutsii* does not grow as well in solution culture with ammonium nitrogen as with nitrate, and that when nitrate is supplied molybdenum is required for optimal growth. The lack of response to molybdenum in the presence of ammonium might have been due to the limiting effects of ammonium on growth.

Apart from their effects on growth, the two sources of nitrogen also appear to affect the absorption of nitrogen and molybdenum, the synthesis of chlorophyll and the levels of free amino acids in the shoots. Asparagine seems to be the principal storage compound for amino nitrogen when ammonium nitrogen is supplied to the plants.

Although molybdenum affects the synthesis of chlorophyll in plants supplied with nitrate, this effect is probably indirect, since no response to molybdenum was obtained in plants receiving ammonium nitrogen. The levels of aspartic acid, glutamic acid, proline, serine, fructose and glucose were also affected by the presence or absence of molybdenum.

It is impossible to say which of the abovementioned effects of molybdenum and the nitrogen source upon the different constituents examined are direct effects and which are indirect.

ACKNOWLEDGEMENTS

This investigation was made possible by a grant from the South African Department of Agricultural Technical Services, for which we are most grateful.

The seed of *Digitaria smutsii* was kindly supplied by the Pasture Research Section of the Agricultural Research Institute, Potchefstroom.

REFERENCES

- AGARWALA, S. C., & E. J. HEWITT (1955a). Molybdenum as a plant nutrient. V. The inter-relationships of molybdenum and nitrate supply in the concentrations of sugars, nitrate and organic nitrogen in cauliflower plants grown in sand culture. *J. Hort. Sci.* 30: 151—162.

- AGARWALA, S. C., & E. J. HEWITT (1955b). Molybdenum as a plant nutrient. VI. Effects of molybdenum supply on the growth and composition of cauliflower plants given different sources of nitrogen supply in sand culture. *J. Hort. Sci.* **30**: 163—180.
- EVANS, H. J. (1956). The role of molybdenum in plant nutrition. *Soil Sci.* **81**: 199—208.
- HEWITT, E. J. (1952). Sand and Water Culture Methods used in the Study of Plant Nutrition. Commonwealth Bur. Hort. Plant. Crops, Techn. Commun. 22. East Malling.
- HEWITT, E. J. (1956). Symptoms of molybdenum deficiency in plants. *Soil Sci.* **81**: 159—172.
- HEWITT, E. J. (1963). The essential nutrient elements: requirements and interactions in plants. In F. C. Steward (Ed.) *Plant Physiology. A Treatise*. Vol. III, pp. 137—360. Academic Press, New York.
- HEWITT, E. J., E. W. JONES & A. H. WILLIAMS (1949). Relation of molybdenum and manganese to the free amino acid content of the cauliflower. *Nature* **163**: 681—682.
- HEWITT, E. J., & C. C. MCCREADY (1956). Molybdenum as a plant nutrient. VII. The effects of different molybdenum and nitrogen supplies on yields and composition of tomato plants grown in sand culture. *J. Hort. Sci.* **31**: 284—290.
- HEWITT, E. J., & A. H. WILLIAMS (1952). Unpublished results supplied by Hewitt (1963), p. 301.
- HUMPHRIES, E. C. (1956). Mineral components and ash analysis. In K. Paech and M. V. Tracey (Ed.) *Modern Methods of Plant Analysis*. Vol. 1, pp. 468—502. Springer-Verlag, Berlin.
- MACKINNEY, G. J. (1941). Absorption of light by chlorophyll solutions. *J. biol. Chem.* **140**: 315—322.
- MININA, E. I. (1960). On the physiological role of molybdenum in plants. *Doklady Akad. Nauk SSSR* **130**: 461—464. (Translated in *Doklady Botanical Sciences* **130**: 32—35).
- MULDER, E. G. (1948). Importance of molybdenum in the nitrogen metabolism of micro-organisms and higher plants. *Plant & Soil* **1**: 94—119.
- NASON, A., & W. D. McELROY (1963). Modes of action of the essential mineral elements. In F. C. Steward (Ed.) *Plant Physiology. A Treatise*. Vol. III, pp. 451—536. Academic Press, New York.
- NICHOLAS, D. J. D., & A. NASON (1954). Molybdenum and nitrate reductase. II. *J. biol. Chem.* **207**: 353—360.
- PARDO, J. H. (1935). Ammonium in the nutrition of higher green plants. *Quart. Rev. Biol.* **10**: 1—31.
- POSSINGHAM, J. V. (1956). The effect of mineral nutrition on the content of free amino acids and amides in tomato plants. I. A comparison of effects of deficiencies of copper, zinc, manganese, iron and molybdenum. *Australian J. Biol. Sci.* **9**: 539—551.
- PRINCE, A. L. (1955). Methods in soil analysis. In F. E. Bear (Ed.) *Chemistry of the Soil*. 1st Ed., pp. 346—347. Reinhold Publishing Corp., New York.
- SNEDECOR, G. W. (1956). Statistical Methods. 5th Ed. Iowa State University Press, Ames.
- STEINBERG, R. A. (1956). Metabolism of inorganic nitrogen by plants. In W. D. McElroy and B. Glass (Ed.) *Inorganic Nitrogen Metabolism*, pp. 153—158. Johns Hopkins Univ. Press, Baltimore.
- STOUT, P. R., & C. M. JOHNSON (1956). Molybdenum deficiency in horticultural and field crops. *Soil Sci.* **81**: 183—190.